Yvette W. Kunz and **C. Wise.** — Development of the photoreceptors in the embryonic retina of *Lebistes reticulatus* (Peters). Electron microscopical investigations. (With 7 figures)

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The viviparous *Lebistes reticulatus* has a diurnal retina. There are four types of cones, which differ morphologically and structurally (MÜLLER 1952). They form a regular mosaic pattern. Moreover they are arranged in three tiers, the innermost (vitreal) and middle layer being made up by single cones, and the outermost (scleral) by twin cones.

One member of each twin cone contains a large globule at the junction between inner and outer segment (MÜLLER 1952). This globule may be either of two types, dense (matrix) or vesicular (BERGER 1966). The vesicular type appears already during embryonic development (KUNZ and WISE 1973). The globule has been termed by BERGER (1966) an oil-globule, similar to those found in amphibian, reptilian and avian photoreceptors. However, histochemical investigations have shown that it does not contain lipids (KUNZ and REGAN 1973). Preliminary ultrastructural analyses suggest that it is a giant, modified mitochondrion (KUNZ and WISE 1973).

One line of our research into this globule has been to establish its embryological origin and subsequent development. To this effect, the developmental pattern of the photoreceptor and adjacent layers had to be established. Electron microscopical analyses were necessary, and the results are reported in this paper.

Material and Methods

Female *Lebistes* were killed, the embryos removed and allocated to the appropriate developmental stages. The embryonic time lasts 30 days at 22° C and is divided into 9 stages (KUNZ 1971). Since pigmentation of the eye appears at stage 5, only embryos from that stage onwards were analysed. The eyes (diameter 0.7-0.8 mm) were fixed and dissected in glutaraldehyde, postfixed in osmium tetroxide and embedded in Araldite. Sections were cut on a LKB III ultrotome, then stained with uranyl acetate and lead citrate and viewed with a Philips 300 electron microscope.

Results

Differentiation in the embryological retina proceeds from the vitreal to the scleral surface, and within each layer from the posterior pole (fundic region) along

all meridians to the rim. The same area within the fundic region—immediately dorsal to the exit of the optic nerve—was chosen for all observations. Only diagnostic characteristics are reported. (Fig. 1 shows a vertebrate photoreceptor with its compartments).

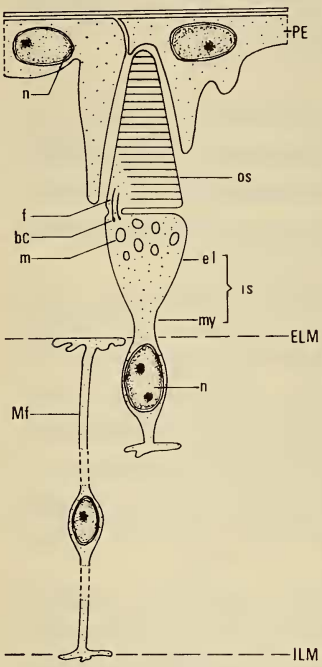

FIG. 1.

Diagram of a vertebrate cone and its compartments, showing its relationship with pigment epithelium and Müller's fibres.

bc	basal corpuscle	MC	middle cone
CG	cristate globule	Mf	Müller's fibre
CH	pigment in chorioid	my	myoid
el	ellipsoid	n	nucleus
ELM	external limiting membrane	os	outer segment
f	filament	PE	pigment epithelium
IC	inner cone	pg	pigment granule
ILM	internal limiting membrane	R	rod
is	inner segment	ss	subsurface membrane
m	mitochondrion	TC	twin cone

Stage 5: This stage can be subdivided into two. *A.* The cuboidal pigment epithelium contains globular granules. Oval nuclei lie with their long axis parallel to the eye surface. *B.* Rod shaped granules have appeared in the pigment epithelium. Inner segments of the photoreceptors start to develop; mitochondria are present in their cytoplasm (fig. 2).

Stage 6: Retinal development takes place in five steps. *A.* Processes at the free surface of the pigment epithelium have begun to form, containing mainly the rod shaped granules. In the ellipsoids basal corpuscles are detectable. Most ellipsoids seem to belong to the twin cones, since subsurface membranes are evident,[1] extending sclerally from the level of the external limiting membrane (fig. 3). *B.* Outer segments begin to form. Filaments are seen to extend into them (fig. 4). *C.* Pigment has appeared in the chorioid (fig. 5). Many of the oval shaped

[1] These are membranes—subsurface and parallel to the plasma-membrane—found in the ellipsoids of both members of the *Lebistes* twin cones (BERGER 1967).

pigmentepithelium nuclei lie now oblique to the scleral-to-vitreal axis. Filaments in the ellipsoids are present. Subsurface membranes in twin cones extend the full length of the ellipsoid. First definition of middle cones is observed. They are at the same level as the twin cones, directly on the external limiting membrane, whereas the rods are in a light adapted position *D*. Mitochondria in both twin- and middle-cone ellipsoids display a scleral-to-vitreal size gradient. In one member of each twin cone the most scleral mitochondrion shows an arrangement of cristae, indicative of a vesicular globule (henceforth called cristate globule). Inner cones can be identified. The ellipsoids of all types of cones lie close to the external limiting membrane (fig. 6). *E*. All nuclei of the pigment epithelium have assumed a radial position (long axis vitreal-to-scleral). Cristate globules, though immature, are present in twin cones. Mitochondria of inner cones display a size gradient, with smallest at periphery and largest centrally placed. Photoreceptor nuclei are arranged in two rows, with the oval cone nuclei sclerally, and the globular rod nuclei vitreally, placed.

Stage 7: Inner, middle and twin cones are arranged in three tiers and are in a light adapted position. Fibrous material has appeared in the cristate globules of the twin cones. Nuclei of middle cones have started to protrude through the external limiting membrane (fig. 7).

It is evident that the major development within the photoreceptor layer takes place within stage 6. Changes in the habitus of the embryo, such as the degree of capillarisation of the pericardial hood (KUNZ 1971), do not proceed parallel with the development of the photoreceptors. It is, therefore, not possible to subdivide stage 6 for our purposes by viewing the embryo in toto. However, subdivision is possible by screening survey sections of the eye, cut on the utltrotome at 1 μ and stained with toluidene blue. This method provides sufficient diagnostic features to identify steps A-E within stage 6.

It is interesting to note that in the embryonic retina the sequence of formation of the diffierent cones in stage 6 is the same as that observed by MÜLLER (1952) in the developing rim of the postembryonic to adult retina.

Problems which emerged during subdivision of stage 6, and which merit detailed analysis at the electron microscopical level are these: 1) Ontogenesis of the cristate globule (see introduction). 2) Development of the subsurface membranes and their relationship to the Müller's fibres (which traverse all the inner layers of the avascular retina). 3) Connection between inner and outer segments of the photoreceptors. This is usually maintained by a ciliary stalk (fig. 1). The distribution of several filaments in the inner as well as outer segment of *Lebistes* cones (fig. 6) suggests an unusual bridging. 4) Embryonic formation of the membraneous discs of the outer segments.

Zusammenfassung

Lebistes reticulatus besitzt eine retina duplex mit vier Sorten Zapfen. Diese sind regelmässig in ein Mosaik und gestaffelt angeordnet. Die embryonale Entwicklung der Sehzellenschicht wird beschrieben, in verschiedene Stadien eingeteilt und mit elektronenmikroskopischen Abbildungen illustriert.

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Planche I

Fig. 2.

Stage 5, B. Pigment epithelium with globular and rod shaped pigment granules. Photoreceptor nuclei in one row. Budding inner segments.
× 4,400

Fig. 3.

Stage 6, A. Mitochondria in developing cone inner segments. Subsurface membranes extending from external limiting membrane.
× 9,600

Fig. 4.

Stage 6, B. Developing outer segments.
× 19,200

Fig. 5.

Stage 6, C. Pigment granules in chorioid. Pigment epithelium with nucleus before rotation.
× 5,500

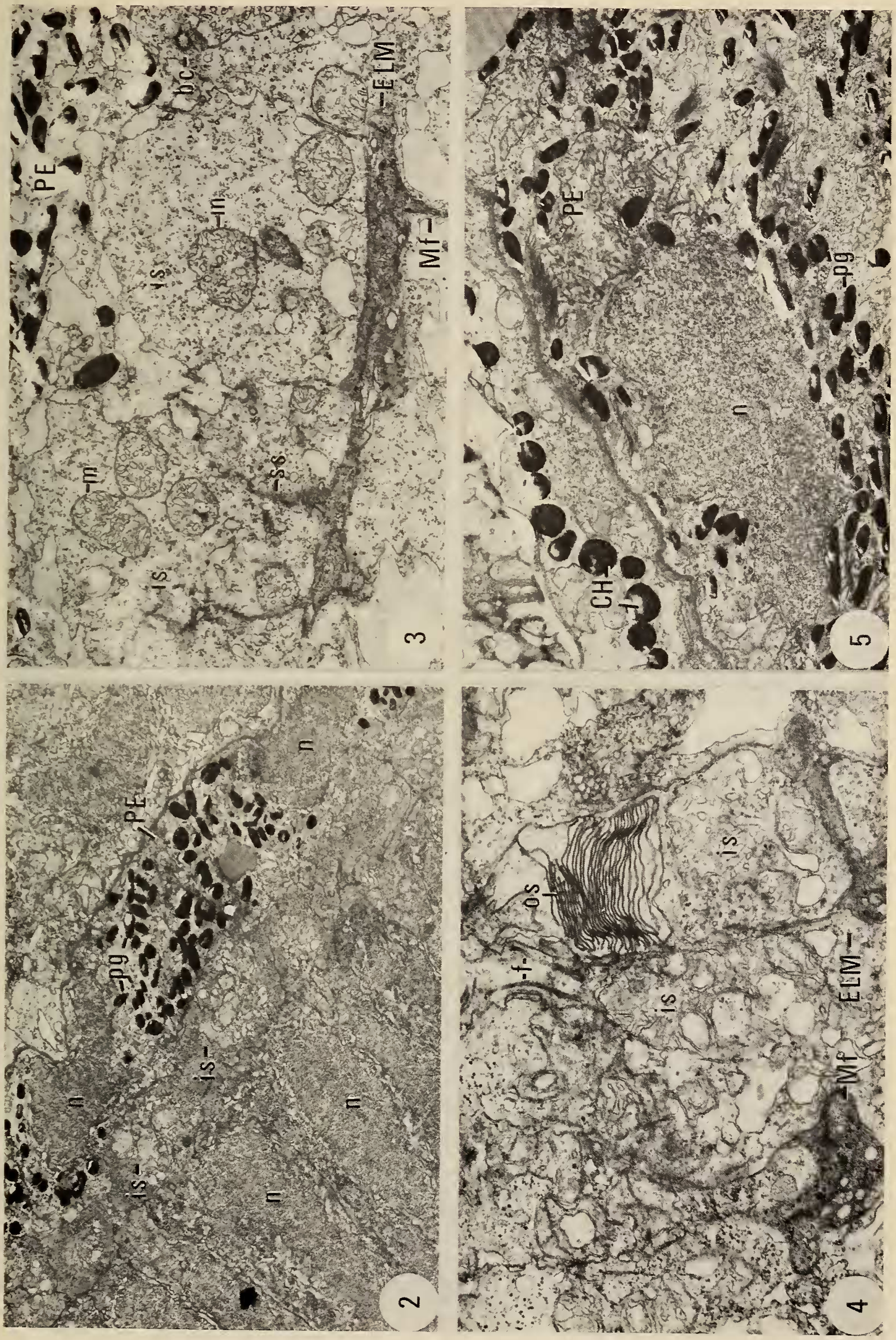
Planche II

Fig. 6.

Stage 6, D. All types of cones present. Mitochondria in middle cones and twin cones with vitreal-to-scleral size gradient. Arrows indicate filaments in inner segments (6 *a*) and outer segments (6 *b*).
× 7,800

Fig. 7.

Stage 7. Cones arranged in three tiers. All photoreceptors in light adapted position.
× 6,900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